CARDIOPROTECTIVE POTENTIAL OF LEAF EXTRACT OF *Andrographis paniculata* IN DOXORUBICIN-INDUCED CARDIOTOXIC ALBINO RATS.

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Abstract

Doxorubicin, sold under the brand name Adriamycin among others, is a chemotherapy medication used to treat different kinds of cancer. Serious side effects may include anaphylaxis, heart damage and tissue damage at injection site. Limited treatment options have made the toxic effects of doxorubicin a serious health challenge. *Andrographis paniculata* also known as 'king of bitters' is an important medicinal plant that is widely used globally. In this study, ethanolic leaf extract of *Andrographis paniculata* at the doses of 50, 100 and 200 mg/kg body weight was investigated for its acclaimed cardioprotective activity in doxorubicin-induced cardiotoxicity in rats. Various biochemical parameters such as Antioxidant enzymes, Reduced glutathione (GSH), Troponin, Lipid profile, Enzyme Biomarkers of tissue damage and Histopathological examination of the heart that relate to cardiotoxicity were assessed using standard methods. The results of the analysis showed that doxorubicin altered the studied parameters of the experimental animals. At two-weeks of intervention with *Andrographis paniculata* leaf extract, it was found to be effective as it ameliorates majority of the changes caused in the parameters of the cardiotoxic rats. The results from this study suggest that *Andrographis paniculata* leaf extract is a potential candidate for the development of an effective drug for the management of heart damage resulting from doxorubicin.

Keywords: *Andrographis paniculata,* Cardiotoxicity, Cancer, Doxorubicin

Introduction

Andrographis paniculata commonly known as king of bitters, creat or green chiretta [1], is an annual herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it has been traditionally used to treat infections and some diseases. It has a myriad of pharmacological properties [2]. The therapeutic potentials of *A. paniculata* have resulted in an increase in its demand. It should be noted that investigation and studies of these various properties needs clinical validation so that they can be used to fight a wide range of diseases, from parasitic infections to cancer.

Doxorubicin, sold under the trade name Adriamycin among others, is a chemotherapy medication used to treat different types of cancer including breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma,

and acute lymphocytic leukemia [3]. It is often used together with other chemotherapy agents and given by injection into a vein [3].

Doxorubicin-induced cardiomyopathy typically results in dilated cardiomyopathy, with all four cardiac chambers being enlarged [4]. This results in both systolic and diastolic dysfunction [4]. Eventually, heart failure can result, which carries a 50% mortality rate. The drug dexrazoxane and ramipril may be used to reduce the risk of doxorubicin's cardiotoxicity in certain cases [4].

Cardioprotection includes all mechanism and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage [5]. This study was designed to evaluate the cardioprotective effect of *Andrographis paniculata* (AP) ethanolic leaf extract in doxorubicin-induced cardiotoxicity in rats.

Materials and Methods

Plant Materials

Fresh leaves of *Andrographis paniculata* (King of bitters*)* were obtained from Iyana Emirin, Ado Ekiti, Ekiti State, Nigeria in September 2022. The harvested leaves of the plant were identified and authenticated at the herbarium of the Ekiti State University in Ado Ekiti.

Reagents and Chemicals

All reagents and chemicals used were of analytical grades.

Animals

Male wistar albino rats weighing between 120-150g, purchased from the Central Animal house of Department of Science Laboratory Technology, The Federal Polytechnic, Ado Ekiti were used for the study. The animals were kept under standard conditions (12h light/12h dark cycle), fed with commercial rat chow (Vital Feeds Nigeria Limited) ad libitum, and liberally supplied with water.

Preparation of ethanolic Extract of *Andrographis paniculata* leaf

Fresh leaves of *Andrographis paniculata* were air-dried for 14 days at room temperature. The air-dried leaves were ground to fine powder using a blender. A 500g of the powdered leaf was soaked in 2000 ml of ethanol for 72 hours, filtered and air-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the refrigerator at 4^0C for further studies.

Experimental Design

Thirty adult male albino rats (Wister strain), weighing between 120-150g divided into six groups (n=6), and designated I-VI were used for the experiment. Groups II –VI were orally administered distilled water, 50 mg/kg b.w *Andrographis paniculata*, 100 mg/kg b.w *Andrographis paniculata*, 200mg/kg b.w *Andrographis paniculata* and 10mg/kg b.w ramipril, respectively for 14 consecutive days while on the 13^{th} day received in addition, single dose of doxorubicin (20mg/kg b.w) intraperitoneally. Group 1 received distilled water only throughout the duration of the experiment and

served as the negative control.

Dissection of Rats

Twenty four hours after the last administration, all animals were anaesthetized with an intraperitoneal injection of 25% urethane and 1% chloralose (5 ml/kg body weight). The rats were dissected and portion of blood was collected into plain bottles for determination of biochemical parameters. The target organ (heart) was excised using scissors and forceps and trimmed of fatty tissues.

Preparation of serum

Serum was prepared by centrifugation at 3000 rpm for 10 min at 25^{0}C. The clear supernatant was collected and used for the estimation of biochemical parameters.

Preparation of Homogenates

The hearts were excised using scissors and forceps. It was washed in buffer solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4°C for 10 mins. The supernatant obtained was collected and stored under 4^{0}C and then used for biochemical analyses.

Enzyme Biomarkers

Assay of Aspartate Aminotransferase (AST) Activity

AST activity was determined following the principle described by [6].

Procedure

Briefly, 0.1 ml of diluted sample of (serum and heart) was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 min at 37^{o}C. 0.5ml of 2,4-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25^{0}C. Then 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance read at 546 nm against the reagent blank after 5 min.

Assay of Alanine Amino transferase (ALT) Activity

The principle described by [6] was followed in the assay of ALT using commercially available assay kit (Randox laboratories, UK) according to the instructions of the manufacturer.

Assay Procedure

Reagent 1 (0.5 ml) containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α-oxoglutarate (2.0 mol/l) was added to 0.1ml of serum and heart in a test tube and the mixture was incubated at 37^{o}C for 30 minutes. Exactly 0.5 ml of R2 containing 2, 4-dinitrophenylhydrazine (2.0 mmol/l) was added and the solution incubated again at 20^{o}C for 20 min. Finally, 5 ml of NaOH was added and the solution was allowed to stand for 5 minutes at room temperature and the absorbance was read at 546 nm.

Calculation

The activity of ALT in the serum was obtained from the standard curve provided in the kit.

Assay of Alkaline Phosphatase (ALP) Activity

Assay of serum ALP was based on the method of [7] using commercial assay kits (Randox laboratories, UK) according to the instructions of the manufacturer.

Assay Procedure

Exactly 1.0 ml of the reagent (1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l p-nitrophenol phosphate) was added to 0.02 ml of the serum and heart samples and mixed. The absorbance was taken at 405 nm for 3 minutes at intervals of 1 minute.

Calculation

ALP activity was determined using the formula U/l = 2760 x A405 nm/min

Lipid Profile Analysis

Estimation of Total Cholesterol Level

Total cholesterol level was determined based on the method of [8] using commercially available kits (Randox laboratories, UK).

Assay Procedure

Standard (10 µl) and serum (10 µl) samples were pipetted into labeled test tubes. 1000 µl of working reagent containing; Pipes buffer (80mmol/l, pH 6.8), 4-Aminoantipyrine (0.25mmol/l), phenol (6mmol/l), peroxidase (≥ 0.5U/ml), cholesterol esteraseion (≥0.15U/ml) and cholesterol oxidase (0.10U/ml)was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 minutes at room temperature. The absorbance of the sample was taken at 500 nm against the reagent blank.

Cholesterol concentration (mg/dl) was calculated as follows:

$$\text{Chol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

Evaluation of Concentration of Triglyceride

Triglycerides level was determined based on the method of [9] using commercially available kits (Randox laboratories, UK).

Assay Procedure

Triglyceride standard (10 µl) and serum (10 µl) were pipetted into labeled test tubes. 1000 µl of the working reagents; R1a (buffer) containing Pipes buffer (40mmol/l, pH 7.6), 4-chloro-phenol (5.5mmol/l), magnesium-ion (17.5mmol/l); R1b (enzyme reagent containing 4-amino phenazone (0.5mmol/l), ATP (1.0 mmol/l), lipase ((≥150 U/ml), glycerol-kinase ((≥0.4U/ml), glycerol-3-phosphate oxidase ((≥1.5U/ml) and peroxidase (≥0.5U/ml)was added into all the tubes. The test tubes were mixed

thoroughly and incubated for 10 minutes at room temperature. The absorbance was taken at 500nm against the blank.

Triglyceride concentration (mg/dl) was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

High Density Lipoprotein (HDL-c)-Cholesterol Assay

The method of [10] was adopted in estimation of the concentration of the HDL- cholesterol in the serum.

Procedure

1. Precipitation

Reaction mixture containing 200 µl of the serum, 200 µl of the cholesterol standard, 500 µl of the diluted precipitant R1 (0.55mM phosphotungstic acid, 25mM magnesium chloride) was mixed together and allowed to stand for 10 min at room temperature. It was then centrifuged for 10 min at 4000 rpm to obtain a clear supernatant. The clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOD-PAP reaction method.

2. Cholesterol CHOD-PAP Assay

To 100 µl of the sample supernatant, 1 ml of cholesterol reagent was added and mixed together in a test tube. The standard test tube contained 100 µl of the cholesterol standard supernatant and 1 ml of cholesterol reagent. The reagent mixture was mixed thoroughly and incubated for 10 min at 25°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was then measured at 500 nm against the reagent blank within 1 h.

Calculation:

Conc. of HDL-Cholesterol = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ X Concentration of standard

Low Density Lipoprotein (LDL) - Cholesterol Determination

The concentration of low-density lipoprotein in the serum was calculated using the formula of [11] as given below:

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL-cholesterol}$$

Very Low Density Lipoprotein (VLDL) - Cholesterol Determination

The concentration of very low-density lipoprotein in the serum was calculated using the formula of [11] as given below:

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

Antioxidant assay

Determination of Catalase Activity

This experiment was carried out using the method described by [12].

Procedure

0.2 ml of samples (serum, pancreas, liver and kidney) was mixed with 0.8ml distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2ml of solution (800μmol) and 2.5ml of phosphate buffer in a 10ml flat bottom flask. Properly diluted enzyme preparation (0.5ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1ml portion of the reaction mixture was withdrawn and blown into 1ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described below.

Calculation

$$\text{Catalase activity} = \frac{\text{H2O2 Consumed}}{\text{mg protein}}$$

H_2O_2 consumed = 800 – Concentration of H_2O_2 remaining

Concentration of H_2O_2 remaining was extrapolated from the standard curve for catalase activity.

Determination of Superoxide Dismutase (SOD) Activity

The level of SOD activity was determined by the method of [13].

Assay Procedure

Sample (1ml) was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Calculation

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5}$$

Where;

A_0=absorbance after seconds

A_3=absorbance 150 seconds

$$\%\ \text{inhibition} = \frac{\text{change in absorbance for substrate}}{\text{Change in absorbance of blank}} \times 100$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

Determination of Reduced Glutathione (GSH) Level

The method of [14] was followed in estimating the level of reduced glutathione (GSH).

Procedure

Exactly 0.2ml of supernatant was added to 1.8ml of distilled water followed by the addition of 3ml of the precipitating solution and then shaken thoroughly. The mixture was then allowed to stand for approximately 5 minutes and then filtered. 1ml of filtrate was added of 4ml of 0.1M phosphate buffer pH 7.4. Finally 0.5ml of the Ellman reagent was added. A blank was prepared with 4ml of the 0.1M phosphate buffer, 1ml of diluted precipitating solution (3parts to 2 parts of distilled water) and 0.5ml of the Ellman reagent. The absorbance was measured at 412nm against reagent blank.

Calculations

The activity of GSH in the serum was obtained from the standard curve.

Determination of GPx

Determination of Glutathione Peroxidase (GPx) Activity

PRINCIPLE:

Glutathione peroxidase (GPX) activity was measured according to the procedure of Mohandas *et al.*, (1984).

$$2\,GSH + H_2O_2 \xrightarrow{\text{Glutathione Peroxidase}} GSSG + 2\,H_2O$$

$$GSSG + \text{ß-NADPH} \xrightarrow{\text{Glutathione Reductase}} \text{ß-NADP} + 2\,GSH$$

Assay Procedure

The reaction solution contained 0.01 ml PMS (10% w/v), 1.58 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml EDTA (0.5 mM, pH 8.0), 0.1 ml sodium azide (1.0 mM), 0.1 ml GSH (1.0 mM), 0.1 ml NADPH (0.1 mM), and 0.01 ml hydrogen peroxide (30%) in a total volume of 2.0 ml. The disappearance of NADPH at 340 nm was recorded for 3 minutes at 30 seconds interval. Enzyme activity was calculated as nmol NADPH oxidized/minute/mg protein using the molar extinction coefficient 6.22×10^3/M/cm.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{rA340nm /min Test - rA340nm / min Blank})(2)(3.1)(df)}{(6.22)\,(0.05)}$$

2 = 2 µmoles of GSH produced per µmole of ß-NADPH oxidized

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm

0.05 = Volume (in milliliters) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

Determination of Total Protein (TP)

The Biuret method described by [15] was employed in the determination of total protein in the serum using commercially available kits (Randox laboratories, UK).

Procedure

1ml of Reagent R1 containing sodium hydroxide (100 mmol/l), Na-K-tartrate (18 mmol/l), potassium iodide (15 mmol/l) and cupric sulphate (6 mmol/l) was added to 0.02 ml of the serum sample, the mixture was incubated at 25ºC and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Calculation

$$\text{Total Protein Concentration} = \frac{A_{sample} \times \text{standard concentration}}{A_{standard}}$$

Histopathology examination on heart

Histopathology examination on the heart was performed according to a modified method of Avwioro (2010) [16].

Results and Discussion

Results

Table 1: Effect of *Andrographic paniculata (*King of Bitters) leaf extract on Heart Biomarkers in Doxorubicin-induced cardiotoxicity in rats

Parameters	ALP (mg/dl)	ALT (U/I)	AST (U/I)	CK-Mb (U/I)	LDH (KUL^{-1})
Non Cardiotoxic Control	16.24 ± 0.53^{b}	27.10 ± 1.02^{ca}	32.17 ± 0.12^{b}	20.84 ± 0.87^{b}	14.55 ± 0.60^{a}
Cardiotoxic Control	30.43 ± 0.29^{f}	42.07 ± 0.68^{f}	49.62 ± 1.21^{e}	34.08 ± 0.53^{f}	26.37 ± 0.52^{e}
Dox + AP (50mg/kg)	24.33 ± 1.02^{e}	36.16 ± 1.13^{e}	45.60 ± 1.32^{d}	27.81 ± 0.96^{e}	22.09 ± 0.75^{d}
Dox + AP (100mg/kg)	20.56 ± 0.82^{d}	34.08 ± 1.20^{d}	37.10 ± 0.43^{c}	25.72 ± 0.52^{d}	18.66 ± 0.59^{c}
Dox+ AP (200mg/kg)	14.03 ± 0.54^{a}	29.62 ± 1.03^{b}	32.92 ± 0.73^{bc}	22.18 ± 0.39^{c}	14.82 ± 0.73^{a}
Dox+ Ram (20mg/kg)	18.26 ± 0.30^{c}	31.42 ± 1.02^{c}	30.20 ± 0.98^{a}	19.73 ± 0.85^{a}	15.28 ± 0.82^{bb}

Values are expressed as mean ± standard deviation (n=5). Values with different superscripts are significantly different at (P<0.05).

KEY:AP= *Andrographic paniculata,* ALP = Alkaline phosphatase, ALT = Alanine amino transferase, AST =Aspartate amino transferase, CK = Creatinine Kinase, LDH=Lactate Dehydrogenase

Table 2: Effect of *Andrographic paniculata (*King of Bitters) leaf extract on Heart Lipid Profile in Doxorubicin-induced cardiotoxicity in rats

Parameters	CHOL(mg/dl)	TRIG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL (mg/dl)	CRI(mg/dl)
Non Cardiotoxic Control	16.07± 0.59^{a}	23.21±0.68^{c}	6.14 ± 0.21^{e}	5.29±0.53^{aa}	4.64±1.07^{aa}	2.61±0.07^{a}
Cardiotoxic Control	29.67 ± 0.42^{f}	36.08± 0.54^{f}	2.53 ± 0.14^{a}	19.92±0.46^{fe}	7.22±0.78^{d}	11.72±0.18^{de}
Dox + AP (50mg/kg)	**25.15±1.14**^{e}	**30.48±1.13**^{e}	**3.87±0.36**^{b}	15.18±0.67^{d}	6.10±0.34^{dc}	6.50±0.19^{dd}
Dox + AP (100mg/kg)	**23.07±0.66**^{d}	**27.66±0.48**^{d}	**4.09±0.20**^{c}	13.45±0.78^{dc}	5.53±0.15^{cb}	5.64±0.70^{cc}
Dox+ AP (200mg/kg)	**17.23±0.43**^{b}	**22.01±0.26**^{cb}	5.39±0.35^{d}	7.44±0.87^{cb}	4.40±0.22^{ba}	3.20±0.87^{bb}
Dox+ Ram (20mg/kg)	19.22±0.62^{c}	21.62±0.44^{a}	7.05±0.28^{ff}	7.85±0.22^{bb}	4.32±0.10^{aa}	2.72±0.46^{aa}

Values are expressed as mean ± standard deviation (n=5). Values with different superscripts are significantly different at ($P<0.05$).

KEY: CHOL–Cholesterol; LDL -Low density lipoprotein; TG-triglyceride; VLDL-Very low density lipoprotein; HDL-High density lipoprotein, CRI-Coronary risk index

Table 3: Effect of *Andrographic paniculata (*King of Bitters) leaf extract on Heart Antioxidant Enzymes in Doxorubicin-induced cardiotoxicity in rats

Parameters	SOD Activity (U/mg protein)	CAT Activity (µmol/min/mg protein)	GSH Concentration (mmole/min/mg protein)	TP (mg protein/ml serum)
Non Cardiotoxic Control	**2.34±0.27^{c}**	**1.42±0.18^{de}**	**4.52±0.20^{e}**	**1.31±0.06^{bc}**
Cardiotoxic Control	**1.87±0.31^{aa}**	**0.84±0.12^{a}**	**2.63±0.24^{a}**	**0.65±0.03^{a}**
Dox + AP (50mg/kg)	**2.08±0.22^{ab}**	**1.18±0.26^{b}**	**3.76±0.27^{b}**	**1.24±0.02^{b}**
Dox + AP (100mg/kg)	**2.10±0.42^{b}**	**1.27±0.14^{c}**	**3.97±0.20^{bc}**	1.29±0.10^{bc}
Dox+ AP (200mg/kg)	**2.17±0.13^{bc}**	**1.37±0.10^{d}**	**4.22±0.20^{d}**	**1.40±0.07^{d}**
Dox+ Ram (20mg/kg)	**2.15±0.20^{bc}**	**1.30±0.26^{c}**	**4.06±0.18^{c}**	**1.36±0.05^{c}**

Values are expressed as mean ± standard deviation (n=5). Values with different superscripts are significantly different at (P<0.05).

KEY: SOD = Superoxide dismutase, CAT = Catalase, GSH = Reduced Glutathione, TP = Total Protein

Table 4: Effect of *Andrographic paniculata (*King of Bitters) leaf extract on Heart Malondialdehyde (MDA) level in Doxorubicin-induced cardiotoxicity in rats

Parameters	MDA/g/tissue
Non Cardiotoxic Control	6.28±0.14^{b}
Cardiotoxic Control	11.41±0.33^{e}
Dox + AP (50mg/kg)	10.59±0.20^{d}
Dox + AP (100mg/kg)	**8.27±0.19^{c}**
Dox+ AP (200mg/kg)	**5.17±0.24^{a}**
Dox+ Ram (20mg/kg)	6.59±0.21^{bc}

Values are expressed as mean ± standard deviation (n=5). Values with different superscripts are significantly different at (P<0.05).

KEY: MDA =Malondialdehyde

Histopathology examination on heart

Representative photomicrographs of the heart of experimental animals showing the histomorphology of the heart.

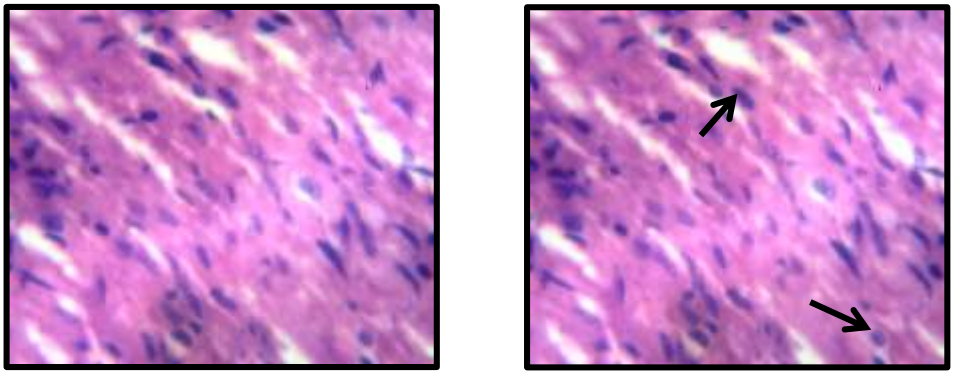

Fig. 1: Non Cardiotoxic Control

Representative photomicrographs of heart of experimental animal showing characteristic staining properties and cellular delineation. The cellularity and morphological delineation apear normal. Black arrow- cardiomyocyte nuclei. X400

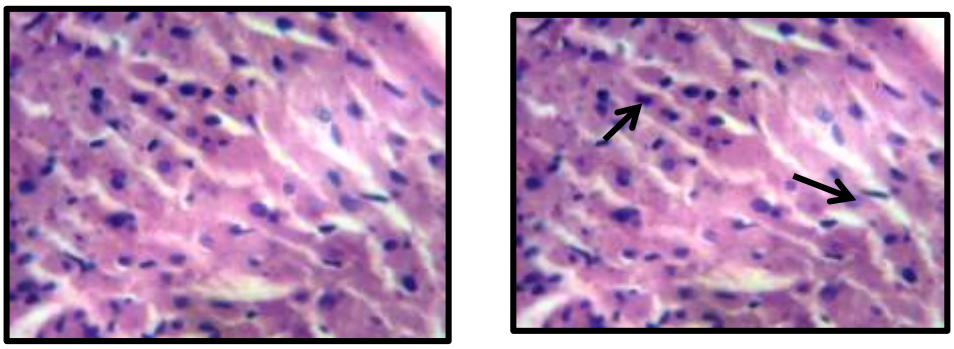

Fig. 2: Cardiotoxic Control: Doxorubicin (20mg/kg) Alone:

Representative photomicrographs of heart of experimental animal showing characteristic staining properties and cellular delineation. The photomicrographs showed marked necrosis, severe infiltration of inflammatory cells and disorganization of myocardium.. Black arrow- cardiomyocyte nuclei. X400

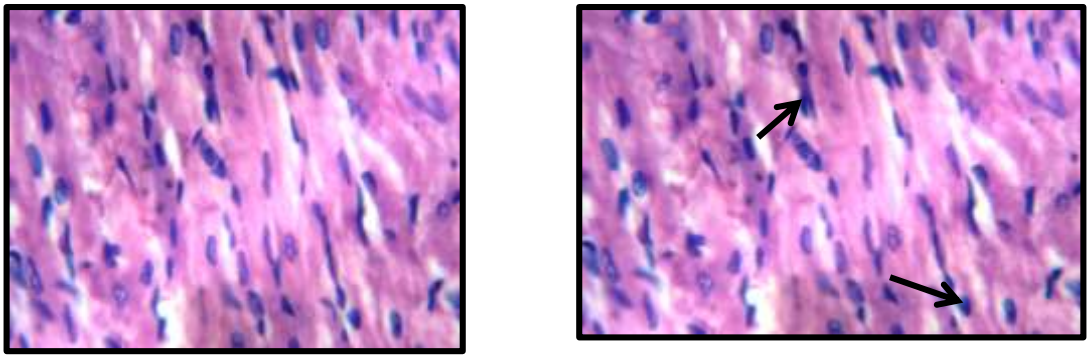

Fig. 3: Doxorubicin(20mg/kg)+100mg/kg *Andrographis paniculata*

Representative photomicrographs of heart of experimental animal showing characteristic staining properties and cellular delineation. The cellularity and morphological delineation appear normal. Black arrow- cardiomyocyte nuclei. X400

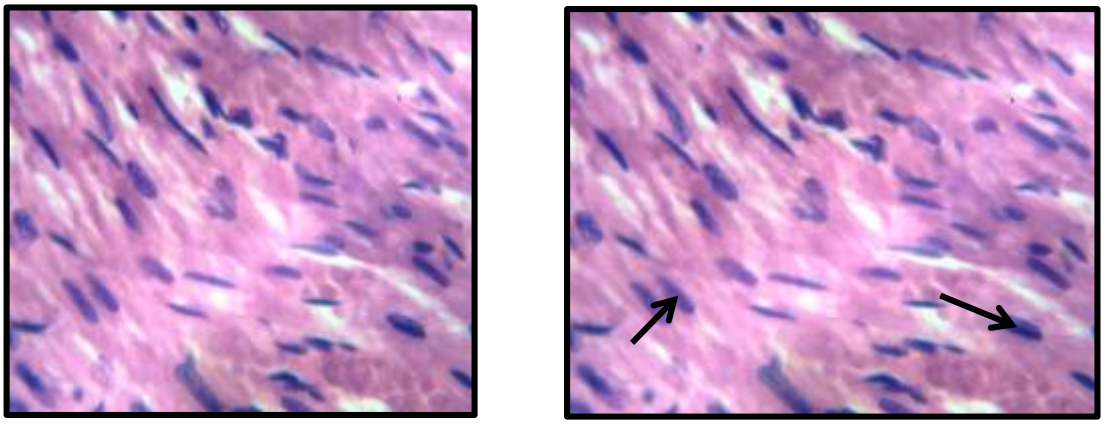

Fig. 4: Doxorubicin(20mg/kg)+200mg/kg*Andrographis paniculata*:

Representative photomicrographs of heart of experimental animal showing characteristic staining properties and cellular delineation.

The cellularity and morphological delineation apear normal. Black arrow- cardiomyocyte nuclei. X400

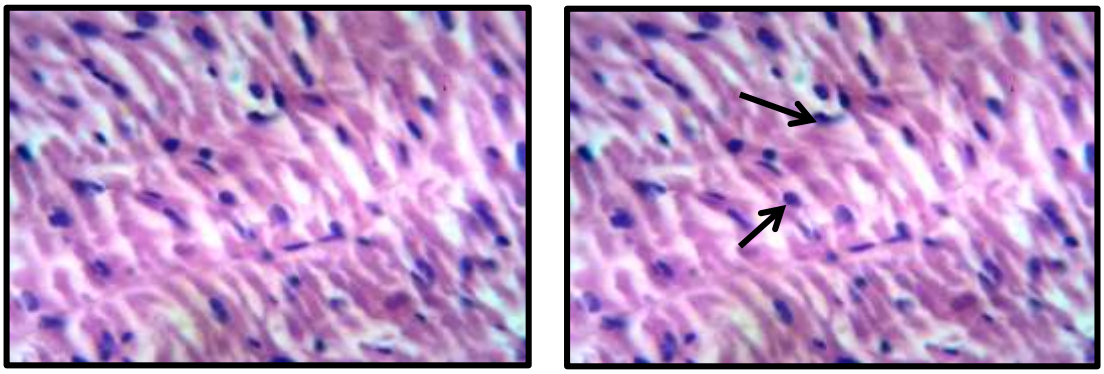

Fig. 5: Doxorubicin(20mg/kg)+10mg/kgRamipril:

Representative photomicrographs of heart of experimental animal showing characteristic staining properties and cellular delineation.

The cellularity and morphological delineation appear normal. Black arrow- cardiomyocyte nuclei. X400

Discussion

The most dangerous side effect of doxorubicin is dilated cardiomyopathy, leading to congestive heart failure. The rate of cardiomyopathy is dependent on its cumulative dose, with an incidence of about 4% when the dose of doxorubicin is 500–550 mg/m^2, 18% when the dose is 551–600 mg/m^2 and 36% when the dose exceeds 600 mg/m^2 [17].

There are several ways in which doxorubicin is believed to cause cardiomyopathy, including oxidative stress, down regulation of genes for contractile proteins, and p53-mediated apoptosis [17]. Doxorubicin-induced cardiomyopathy typically results in dilated cardiomyopathy, with all four cardiac chambers being enlarged [18]. This results in both systolic and diastolic dysfunction [18]. Eventually, heart failure can result, which carries a 50% mortality rate. The drug dexrazoxane may be used to decrease the risk of doxorubicin's cardiotoxicity in certain cases [18]. Once the cardiomyocytes were injured because of doxorubicin administration, this would be followed by disruption of associated cell membranes, and these intracellular proteins will then be released into the circulation, promptly increasing the serum levels of these enzymes during the acute phase of necrosis.

Andrographis paniculata has long been used in traditional medicine for the treatment and prevention of various ailments. The active constituent of *A. paniculata* is andrographolide; its medicinal properties have been reported [19].

Detection of injury in a cell, tissue or an organ could be afforded by measuring levels of known marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatine kinase (CK) activity [20].

In this study, it was observed that administration of doxorubicin caused cardiac damage as it was evident in the significant increase in the activity of cardiac biomarkers (AST, ALP, ALT, CK and LDH) in rats treated with doxorubicin.

The increase of cellular enzymes reflects the alterations in cardiac membrane integrity and/or permeability as a response to α-adrenergic stimulation.

Doxorubicin administration produces free radicals via adrenoceptor mechanism and affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis [21].

Ethanolic leaf extract of *Andrographis paniculata* decreased the activities of these enzymes in the heart. This could be because of the protective effect of ethanolic leaf extract of *Andrographis paniculata* on the myocardium, thus reducing the cardiac damage thereby reducing the activity of these enzymes.

An increase in concentration of total cholesterol and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction [22]. High level of circulating total cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage [22]. Administration of doxorubicin raised LDL cholesterol and decreased HDL cholesterol level in the heart. *Andrographis paniculata* elevated HDL level and decreased LDL cholesterol level.

Hypertriglyceridemia was observed in doxorubicin treated rats and it is clinically reported in ischemic heart disease. Pretreatment with *Andrographis paniculata* prevented the elevation of triglycerides. Prevention of elevation of cholesterol, triglyceride and LDL in heart signify that the myocardial membrane is intact and not damaged.

Antioxidant enzymes, mainly SOD and CAT are the first line of defense against free radical induced oxidative stress eliminating reactive oxygen radicals such as superoxide anion and hydrogen peroxide, and preventing the formation of more reactive hydroxyl radical [23].

SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide, and CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water [23].

A decrease in the level of antioxidant enzymes was recorded after doxorubicin intoxication. Damage of cardiac tissue observed in the present study may result from the decrease of antioxidant enzymes in the heart following exposure to doxorubicin.

Doxorubicin caused a significant decrease in the activities of SOD and CAT. The *A. paniculata* leaf extract treated groups demonstrated attenuating effects against the doxorubicin-induced oxidative stress. This indicates that the plant has potent antioxidant activities; a finding that supports existing report of Yerra et al., 2006 [24]. Damage of cardiac tissue observed in the present study may result from the decrease of antioxidant enzymes in the heart following exposure to doxorubicin. The *A. paniculata* leaf extract treated groups demonstrated attenuating effects against the doxorubicin-induced oxidative stress.

GSH, protein thiol and non-protein thiol are non-enzymatic defences, which are important for maintaining cell integrity because of their reducing properties [25]. The function of GSH and thiols is to serve as the reductant of toxic peroxides. When there is deficiency or depletion of GSH, it causes damage to the macromolecules or membranes. The deficiency of GSH caused by doxorubicin indicates its interaction with biomembrane and subsequent peroxidising action. The inhibitory effect on depletion of GSH levels by pre-treatment with *A. paniculata* in rats further revealed the protective effect of this extract on doxorubicin-induced MI, and this effect may be because of the antioxidant property of *A. paniculata* [26].

In this study, rats pre-treated with *Andrographis paniculata* leaf extract showed increased activities of these antioxidant enzymes, which strongly suggested that *Andrographis paniculata* has the ability to mitigate the deleterious effects of free radicals in doxorubicin-induced rats in agreement with the previous report by Sivakumar and Rajeshkumar, (2015) which indicated that *A. paniculata* enhanced antioxidant enzymes [27].

Histopathological changes of the cardiac tissue manifesting as, marked necrosis, severe infiltration of inflammatory cells and disorganization of myocardium was observed following the induction of doxorubicin. Severe histoarchitectural distortion of the cardiac cells observed as marked necrosis, severe infiltration of inflammatory cells and disorganization of myocardium in the heart section of Wistar rats treated with

doxorubicin only when compared with the heart sections of the control is indicative of doxorubicin related cardiotoxicity. Necrosis is a type of cell death that occurs after abnormal stresses such as chemical injury or toxin. Necrotic cells are unable to maintain membrane integrity as they leak out their content and this may elicit inflammation in the surrounding tissue [28]. This result is in accordance with reports on CCl_4 administration namely, loss of the normal liver histoarchitecture and cytotoarchitecture [29]; [30]; [31].

Treatment with *Andrographis paniculata* leaf extract at (100 mg/kg and 200 mg/kg) doses followed by the administration of doxorubicin revealed histoarchitectural preservation of the heart against doxorubicin intoxication.

It therefore implies that *Andrographis paniculata* restores homeostasis of the antioxidant system by conjugating and excreting toxic cellular molecules, detoxification of ROS, sustenance of cellular integrity as well as a possible enhancement of tissue regeneration.

Conclusion and Recommendation

The present work showed that ethanolic leaf extract of *Andrographis paniculata* has a protective effect on doxorubicin-induced cardiotoxicity as evidenced biochemically. The extract donated its electrons to free radicals thereby preventing the free radicals from being toxic.

It is recommended that the leaf of *Andrographis paniculata* should be explored as an adjunct therapy to ameliorate the cardiotoxicity induced by the use of doxorubicin in cancer treatment. The plant is very bitter and can be air-dried, milled and put in capsule as a supplement.

References

[1] Hossain MS, Urbi Z, Sule A, Hafizur Rahman K. M (2014): "Andrographis paniculata (Burm.f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology "*ScientificWorldJournal* 1–28.

[2] Peter Tzemis and Laura Magnifico, (2014): “The benefits and side effect of *Andrographis paniculata* (Kalmegh)”, Body Nutrition. Last updated: July 15, 2016.

[3] Brayfield, A, (2013): "Doxorubicin". Martindale: The Complete Drug Reference Pharmaceutical Press. Retrieved 15 April 2014.

[4 Kanu C; Jianqing Z; Norman H; Joel S. and Karliner (2010). "Doxorubicin Cardiomyopathy". *Cardiology.* **115** (2): 155–162.

[5] Kubler W, and Haass M. (2000): Cardioprotection: definition, classification and fundamental principles. *Heart*; 75:330-33

[6] Reitman S. and Frankel S, (1957): *Amer. J. Clin. Path.*,28:56.

[7] Englehardt A. *Et al.,* Aerztl Labor. (1970): 16 42.

[8] Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry;* **6**: 24-27.

[9] Tietz, N.W. (1990): Clinical Guide to Laboratory Tests. Second Edition W.B. Saunders Company, *Philadelphia*, USA 554– 556.

[10] Grove, T.H. (1979): Effects of reagent pH on the determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clinical Chemistry*. **25**: 560-564.

[11] Friedwald, W.T., Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the concentration of low density lipoprotein cholesterol in the plasma, without the use of preparative ultracentrifuge. *Clinical Chemistry*; **18**: 499-502.

[12] Sinha, A. K. (1972): Colorimetric assay of catalase.*Journal of Analytical Biochemistry*; **47**: 389 - 394.

[13] Misra, H. P., and Fridovich I. (1972): *Journal of Biological Chemistry*. ASBMB. 33: 286 -293.

[14] Beutler, E., Duran, O. and Kelly, M.B. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory Clinical Medcine*; **61**: 882-890.

[15] Weichselbaum, R.R. and Hellman, S. (1995): Oligometastase. *Journal of Clinical Biochemistry*; 13: 8-10.

[16] Avwioro O. G. (2010): Histochemistry and tissue pathology, principle and techniques, Claverianum press, Nigeria.

[17] Chaterjee, K.; Jianqing Z.; Norman H.; Joel S. K (2010): "Doxorubicin Cardiomyopathy".*Cardiology.* 115 (2): 155– 162.

[18] Kanu C; Jianqing Z; Norman H; and Joel S. (2010): "Doxorubicin Cardiomyopathy". *Cardiology.* **115** (2): 155–162.

[19] Tan, W.D., Peh, H.Y., Liao, W., Pang, C.H., Chan, T.K., Lau, S.H. *et al.*, (2016): 'Cigarette smoke-induced lung disease predisposes to more severe infection with nontypeable *Haemophilus influenzae*: Protective effects of andrographolide', *Journal of Natural Products* 79(5), 1308– 1315.

[20] Dianita, R., Jantan, I., Amran, A.Z. and Jalil, J., (2015): 'Protective effects of *Labisia pumila* var. *alata* on biochemical and histopathological alterations of cardiac muscle cells in isoproterenol-induced myocardial infarction in rats', *Molecules* 47, 4746–4763.

[21] Upaganlawar, A. and Balaraman, R., (2011): 'Cardioprotective effects of *Lagenaria siceraria* fruit juice on isoproterenol- induced myocardial infarction in Wistar rats: A biochemical and histoarchitecture study', *Journal of Young Pharmacists* 3, 297–303.

[22 Al-Yahya, M.A., Mothana, R.A., Al-Said, M.S., El-Tahir, K.E., Al- Sohaibani,M. and Rafatullah, S., (2013): 'Citrus medica Otroj: Attenuates oxidative stress and cardiac dysrhythmia in isoproterenol- induced cardiomyopathy in rats', *Nutrients* 5(11), 4269–**4283.**

[23] Sawyer, D.B., Siwik, D.A., Xiaol, L., Pimentel, D.R., Singh, K. and Colucci, W. S., (2002): 'Role of oxidative stress in myocardial hypertrophy and failure', *Journal of Molecular and Cellular Cardiology* 34 (4), 379–388.

[24] Yerra, L., Anavekar, N., Skali, H., Zelenkofske, S., Velazquez, E., McMurray, J. *et al*., (2006):'Association of QRS duration and outcomes after myocardial infarction: The VALIANT trial', *Heart Rhythm* 3(3), 313–316.

[25] Hadi, S., Mirmiran, P., Hadi, V., Hosseinpour-Niazi, S. and Emamat, S.M., (2016): 'Effects of *Nigella sativa* on Cardiovascular Risk Factors', *Journal Medical Science* 25(134), 430–442.

[26] Hassan, S. M., Khalaf, M. M., Sadek, S.A. and Abo-Youssef, A.M., (2017): 'Protective effects of apigenin and myricetin against cisplatin-induced nephrotoxicity in mice', *Journal of Pharmaceutical and Biomedical Analysis* 55(1), 766–774.

[27] Sivakumar, V. and Rajeshkumar, S., (2015): 'Protective effect of *Andrographis paniculata* on hyperglycemic mediated oxidative damage in renal tissues of diabetic rats', *Journal of Phytopharmacology* 4(6), 287–294.

[28] Kumar V, Alla SR, Krishnan KS, and Ramaswami M. (2009): Syndapin is dispensable for synaptic vesicle endocytosis at the Drosophila larval neuromuscular junction. *Mol Cell Neurosci*;40:234-41.

[29] Prakash T, Fadadu SD, Sharma UR, Surendra V, Goli D, Stamina P, (2008): Hepatoprotective activity of leaves of *Rhododendronarboreum* in CCl_4 induced hepatotoxicity in rats. *J Med Plants Res* ;2:315-20.

[30] Soufy NI. (2012): Hepatoprotective and antioxidant effects of *Silybummarianum* plant against hepatotoxicity induced by carbon tetrachloride in rats. *J Am Sci*;8:479-786.

[31] Padhy BM, Srivastava A, Kumar VL. (2007): *Calotropisprocera* latex affords protection against carbon tetrachloride induced hepatotoxicity in rats. *J Ethnopharmacol* 113:498-502.